DEVELOPMENT, PHYSICOCHEMICAL CHARACTERIZATION, AND *IN VITRO* SAFETY PROFILE OF NANOCAPSULES CONTAINING EPICATECHIN

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INTRODUCTION: Epicatechin, a flavonoid found in natural products, has antioxidant and anti-inflammatory properties, but its clinical use is limited by low bioavailability and instability. Nanotechnology appears as a promising alternative to protect this molecule and enhance its effectiveness. OBJECTIVE: to develop and characterize nanocapsules containing epicatechin (NC-EC) in terms of physicochemical parameters and evaluate their *in vitro* safety profile. MATERIALS AND METHODS: Nanocapsules (NC) were prepared by interfacial deposition of the preformed polymer method. The organic phase was composed of Eudragit L-100, crodamol, epicatechin, and sorbitan monoesterate and dissolved in ethyl alcohol. The organic phase was poured into the aqueous phase containing polysorbate 80 and ultrapure water. The organic solvent was evaporated using a rotary evaporator to a final volume of 20 mL and 0.25 mg/mL of epicatechin. Blank NCs (NC-B) were produced without the addition of epicatechin. Finally, the NCs were characterized according to average particle size, polydispersity index (PDI), Zeta potential (ZP) and pH. VERO cells were used to conduct the *in vitro* safety profile. The cells were plated in 96-well plate ($1x10^5$ cells/well) and exposed to a concentration curve of NC-EC and NC-B (0.01 to 50 ug/mL) during 24, 48 and 72h of incubation. Cell viability and proliferation indexes, nitric oxide (NO) production, reactive oxygen species (ROS) levels and dsDNA release were assessed. For statistical analysis, the results were presented as a percentage and one-way ANOVA was performed followed by Tukey's post hoc. RESULTS AND CONCLUSION: The NC-EC presented adequate physicochemical characteristics, with an average size of 164.7 nm, PDI of 0.162, ZP of -9 mV and pH of 3.85. The NC-B showed similar parameters. *In vitro* tests revealed that NC-EC and NC-B concentrations from 0.01 to 1 ug/mL did not alter the cellular homeostasis, however, concentrations of both formulations of 10 and 50 ug/mL were cytotoxic, especially after 24 and 48h of exposure. Obtained results showed that NC-EC presented nanometric size, homogeneity, and characteristics compatible with the excipients. The *in vitro* safety profile was satisfactory, and the cytotoxicity seems to be related to the intrinsic characteristics of the NC and not to the presence of epicatechin.

Keywords: Nanotechnology; Nanotoxicology; Flavonoid.